Survey of Phytoparasitic Nematodes in Madhya Pradesh and Taxonomy of Certain Tylenchids.

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C O N T E N T S

INTRODUCTION

During the last quarter of the century or so, the science of plant nematology has increased considerably. It has been proved through pathogenicity studies that many of them cause considerable loss to the cultivated crops (Southey and Samuel, 1954; Cairness, 1955; Allen and Maggenti, 1959; Hutchinson et al., 1961; Taylor et al., 1967; van Berkum and Seshadri, 1970). Many of them in association with certain other component of soil biota enhance the disease syndrome and hence crop losses (Powell, 1963, 1971; Pitcher, 1965; Miller, 1965;). Recently the study of phytoparasitic nematodes has attained still greater significance because some genera belonging to the order Dorylaimida such as Xiphinema spp., Longidorus spp. and Trichodorus spp. have been proved to be vectors of both tubular (NETU) and polyhedral (NEPO) viruses (Hewitt et al., 1958; Harrison and Cadman, 1959; Harrison et al., 1961; Walkinshaw et al., 1961; Fulton, 1962; Gibbs and Harrison, 1964).

By the end of 1966 more than one thousand species belonging to phytoparasitic group had been described (Allen and Sher, 1967). Indian nematologists had, in the last decade or so, made a significant contribution (Ahmad, 1972; Chawla et al., 1968; Das, 1960; Edward and Misra, 1963,

1965, 1969; Edward et al., 1965; Fotedar and Mahajan, 1973, 1974; Husain and Khan, 1965, 1967, 1968; Jairajpuri, 1963, 1965; Jairajpuri and Baqri, 1973; Jairajpuri and Siddiqi, 1969; Khan and Basir, 1963; Khan and Hussain, 1965; Khan and Nanjappa, 1970; Khan et al., 1967; Khan and Siddiqi, 1968; Khan and Singh, 1974; Muddassirul Mulk and Jairajpuri, 1974; Rashid and Khan, 1972; Siddiqi, 1959, 1960, 1961,1962, 1963, 1964, 1966, 1972; Siddiqi and Brown, 1965; Siddiqi and Goodey, 1963; Siddiqi et al., 1963; Siddiqi and Hussain, 1964; Singh, 1971; Swarup and Sethi, 1968). In most of these cases, however, the concept of morphological type species had been taken into consideration. Such a concept has its own importance, however, for the advisory work for the conrol of phytoparasitic nematodes, this is not enough. It is highly desirable to know what kind and numbers of phytoparasitic nematodes comprise the existing population and how much damage such population may cause to the crop (Barker and Nusbaum, 1971) as it had been observed that many of these morphological species show a wide range of variation in morphology and dimensions (Coomans, 1963; Bird and Mai, 1965; Brzeski and Zuckerman, 1965; Tarjan, 1967; Geraert, 1968) which according to some taxonomist is the function of geographical isolation, ecological stresses and the genetic inequality.

In view of the above, it is proposed to study the following aspects:-

1. Survey of phytoparasitic nematodes from the soil around the roots of vegetables, fruit trees and other ornamental plants in certain districts of Madhya Pradesh.

2. Description and diagnosis of new form of phytoparasitic nematodes if any, in the group Tylenchida.

3. Variation and variability of morphological and morphometric characters in Hoplolaimus indicus, Helicotylenchus retusus and Tylenchorhynchus brassicae as influenced by ecological stresses, such as:

   a. population levels.
   b. sexrations
   c. soil types
   d. soil moisture levels and
   e. hosts.

REVIEW OF LITERATURE

Surveys of phytoparasitic nematodes had been carried out by a number of scientists working in different laboratories (Prasad et al., 1964; Swarup et al., 1964; Saxena and Hussain, 1969; Sethi and Swarup, 1969; Yadav et al., 1969; Rashid et al., 1973; Mukhopadhyaya and Haque, 1974). By andlarge, the surveys made so far reported the nematode fauna of areas located near some active centres of plant nematology or at best in relation to a particular crop (Prasad et al., 1964; Singh et al., 1964; Singh and Misra, 1974) or to a particular nematode (Seshadri and Sivakumar, 1963; Mukhopadhyaya et al., 1968; Koshy and Swarup, 1971; Khan et al., 1972). Invariably, all of them failed to indicate variations and variability of these nematodes encountered by them. Variation is a common feature of many of the nematodes. (Thorne and Allen, 1959; Allen and Sher, 1967).

It was reported for the first time by Godfrey (1929), the intra-specific variation in the shape of tail of Pratylenchus brachyurus where it ranged from rounded to square terminus in a naturally occurring population. However, no mention was made of the relative frequency of

these variations. Chitwood (1957) warned the taxonomists for giving more weight to size of nematodes for it depended on age, physical conditions and the food supply etc. Since then intra-and inter-specific differences had been observed in several nematodes (Taylor and Jenkins, 1957; Bedding, 1968; Roman and Hirschmann, 1969). This subject had been discussed in great detail by Hooper (1969), Franklin (1970), Loof (1970) and Sturhan (1970). Thorne and Allen (1959), considered that although measurements of individual nematodes in natural population vary greatly according to their stage of development a deviation of 10-35% of mean might be due to genetic variation. Coomans (1971) attributed them to the differences in the genetic constitution and or differences in the environment.

DeConinck (1962), while discussing the problems of systematics and taxonomy of nematodes in relation to various characters such as: form of the tail and lip region, the size of eggs or of the spicules, the number of pre-and/or caudal papillae; expressed that it might be an expression of specific diversity; or intra-specific, phenotypical or genetic variability or might be due to the effect of ecological and geographical factors. This led him to conclude that biological species concept in case of many nematode species does not hold good due to lack of data on potential interbreeding.

Various populations of nematodes, such as: Pratylenchus vulnus (Sher and Allen, 1953); Rotylenchus goodeyi and Hoplolaimus pararobustus (Coomans, 1962, 1963); Hetrodera glycines (Golden and Epps, 1965; Miller and Duke, 1967); Rhabdontolaimus carinthiacus (Blinova-Lazarevskaya and Kakuliya, 1967); Rotylenchulus reniformis (Ching, 1969); Macroposthonia xenoplax , M. sphaerocephala and Criconemoides informis (Grisse, 1970); Aphelenchus agricola (Sigareva and Krasnopolskii, 1974); Belonolaimus longicaudatus (Robbins and Hirschmann, 1974); Xiphinema krugi (Frederick and Tarjan, 1975), exhibited intra-specific variations in the morphology and dimensions. However, Yeates (1972), observed that dimensions of Tylenchus leptosoma , Plectus parietinus , Tylencholaimus mirabilis and Mononchus mirabilis , in a given locality remained unaffected. In case of many others, morphometric differences led scientists to synonymize nematode species. As for example: Goodey and Hooper (1965), synonymized Metaphelenchus rhopolocercus and M. micoletzkii to Aphelenchus avenae ; Metlitski (1968), Ditylenchus fragariae , D. allii , D. floxidis , D. trifolii and D. destructor to D. dipsaci ; Baqri and Jairajpuri (1970), Tylenchorhynchus dactylurus , T. digitatus , T. crassicaudatus , T. elegans and T.zeae to T.mashhood

Population of various geographical orgin, exhibit morphological and morphometric variations (Bird, 1966; Bird and Mai; Tarjan, 1969; Heyns and Van Ark, 1973; Mamberti, 1973) Tarjan (1969), observed that the populations of Xiphinema americanum obtained from equatorial regions with higher temperature and precipitation were shorter in length, with smaller "a" ratio, a wider range of "c" ratio, stylet lengths and lower vulva percentage. Similarly, the populations of Longidorus africanus , obtained from different geographical regions, led Lamberti (1973) to divide into three groups. "The East African type" from Rhodesia and Somali land and 2.8-3. 1mm long; "the North African type" from Egypt and Sudan and 4.1-4.5mm long with an Israeli population (4mm long); and "the Californian type" intermediate with length 3.8-3.9mm with Mexican population (3.5mm long).

Not only the populations, individuals also, derived from single gravid female, showed variations in morphology and dimensions (Van Weerdt, 1958; Sanwal and Loof, 1967; Tarjan and Frederick, 1974; Chin, 1975). Allen (1952) observed a high degree of variation in the perineal pattern of root-knot nematode Meloidogyne incognita acrita and M. hapla and in the number of labial annules in males of latter which varied from 0 to 3.

The existence of polymorphic species in nematodes was revealed by Hirschmann (1951) in a diplogasterid (Pristonchus lheritieri) with a stenostome and an eurystome form. Hirschmann (1952), while studying the population of Ethmolaimus pratensis deMan observed a great variation in the position of the amphid with respect to the stoma. This led him to synonymize 9 species of the genus to this species. Subsequently this was observed later in Hemicycliophora zuckermani by Brzeski (1963), Brzeski and Zuckerman (1965) and Minton and Golden (1966), Further, Minton and Golden (1965) reported a continuous tail shape variability in an undescribed species of Hemicycliophora. Heteromorphism in tail shape in Hoplolaimus larvae (Minton and Golden, 1968; Golden and Minton, 1970) and adult females (Gupta and Edward, 1974) had been reported.

Among the factors which have so far been attributed in influencing the nematode morphology and domensions are physical conditions of the soil, soil biota, host plant, nutrition, population of nematodes and the sex ratio.

The morphometric characters of nematodes such as length; width; spear length; gonad length; ratios "a", "b" and "c" were influenced by soil temperature. In Paratylenchus nanus (Fisher, 1965), Ditylenchus destructor

and Ditylenchus myceliophagus (Evans and Fisher, 1970), most of these values decreased at 15°c or above. In Panagrellus silusiae (Gysels, 1964), on the other hand, the length of the nematode decreased with an increase in temperature. Rohde and Jenkins (1957) and Malek and Jenkins (1964) observed that length and width of Trichodorus christiei was inversely related to the temperature. This was, however, not true with the position of vulva in Paratylenchus nanus , Ditylenchus myceliophagus and D. destructor (Fisher, 1965; Evans and Fisher, 1970) and the length of post vulval sac (Evans and Fisher, 1970). Males of Paratylenchus nanus, in general, were less sensitive than the females but at higher temperature reverse was true (Fisher, 1965).

Morphology and dimensions of various nematodes such as Ditylenchus myceliophagus (Goodey, 1958); Ditylenchus triformis, Ditylenchus sp., Neotylenchus linfordi , Aphelenchus avenae , Paraphelenchus sp. (Pillai and Taylor, 1967); Aphelenchus rutgersi (Hooper and Meyrs, 1971), Aphelenchus avenae (Monson, 1971; Townshend and Blackith, 1975) were influenced when reared on various soil inhabiting fungi. Similary certain bacteria influenced the taxonomic features of Acrobeloides uberrinus (Anderson, 1965); Acrobloides sp. (Anderson, 1968);

Acrobeloides nanus (Sohlenius, 1973). Even bacterial growth factor when supplemented in the culture medium brought about similar changes in Caenorhabditis briggsae (Kiesiel, et al., 1969).

Ludwig (1938), Stephenson (1942), Paetzold (1958) and Myers (1967a) reported that even different culture media employed to cultivate various nematodes influenced their morphologic and morphometric features.

Taxonomic features in nematodes varied depending upon the suitability of the host plant (Goodey, 1952; Wu, 1960; Chitwood, 1962; Bird and Mai, 1965; Brzeski, 1967; Behrens, 1974; Cook, 1975). Metliski (1969), observed that in Ditylenchus dipsaci , body size and proportions were influenced to such an extent that they could not be used in separating various races in this nematodes. Resistant hosts often decreased mean body and stylet lengths in larvae and males of Heterodera rostochiensis (Trudgill and Parrott, 1970). Even in case of Aphelenchus avenae (Monson, 1971) and Acrobeloides nanus (Sohlenius, 1973) length of the nematodes depended on the kind of food supplied to them. However, the relative position of vulva in Ditylenchus destructor (Goodey, 1952) and the length of sylet and spicule in Heterodera rostochiensis pathotype

A and H. pollida pathotype E. (Behrens, 1974) remained practically unaffected. Moreover, stylet length in Trichodorus christiei (Bird and Mai, 1965) and spicule length in Rhabditis teres (Ludwig, 1938) on the other hand were quite variable. Females, in general were more sensitive than males (Brzeski, 1967). Physiological status of the host also influenced these features (Rühm, 1962, Sanwal, 1965; Evans and Fisher, 1970; El-Sherif, 1972).

The nematode number and sex ratios did not appear to influence significantly the average length of body and stylet of Paratylenchus nanus , however, position of vulva was displaced towards posterior side with the increse in number of nematodes (Fisher, 1965). High population density of Heterodera schachtii , on the other hand, brought about shortening in the length of their males (Kerstan, 1971)

Techniques employed for processing (Stone, 1971) or for permanent mount preparations (Geraert, 1961; Diab, 1965) also brought about certain dimensional changes in nematodes.

In taxonomic studies deManian ratios are used by convention. But, because these ratios are formed by two variables and are therefore, approximation (Clark, 1962)

and hence should be used with caution (Brzeski and Szczygiel, 1963; Diab, 1965; Geraert 1968, Fisher, 1969). In view of this, the use of statistical methods in nematode taxonomy has been suggested (Barraclough and Blackith, 1962; Angervall and Carlstrom, 1963; Rau and Fassuliotis, 1966; 1967; Bird, 1967b; Terenteva, 1967; Bird and Mai, 1968; Roggen and Asselberg, 1971; Tobar Jimenez, 1971).

MATERIALS AND METHODS

Collection of soil samples:

Soil and root samples from vegetables, fruit trees and ornamental plants from various localities in Madhya Pradesh will be collected with the help of metallic sampler (Prod) or shovel. Each soil sample weighing approximately 1kg will be taken up from the depth of 10-30mm in case of vegetables and ornamental plants and 20-50cm or more in case of fruit trees. From agricultural fields, soil will be sampled randomly and mixed up thoroughly. From this composite sample, approximately 1kg of soil will be taken. Soil and root samples will be collected in polythene bags. Aluminium tags indicating host, locality, soil type, and date of collection will be kept with each sample. Each bag will be sealed with rubber band. Nematodes will be isolated from each samples at the spot if possible, otherwise will be brought to the laboratory and stored in refrigerator at 5-10°c till isolation is done.

Processing of soil samples:

Soil will be processed by Cobb's (1918) modified seiving and decanting technique. Desired quantity of

soil sample will be taken in plastic bucket. It will be half filled in tap water and stirred gently to break the soil crumbs. After few minutes of stirring, the suspension will be left undisturbed for few minutes to allow the heavier soil particles to settle down. Coarse debris will be removed by pouring the aliquot through 50 mesh sieve in another bucket. This aliquot will be poured through 240 and 350 mesh sieves. The catch from these sieves will be collected in a beaker. Suspension from the beaker will be poured over tissue paper mounted on a coarse supporting sieve placed into Baermann funnel. Funnel will be filled up with the water, if desired, until it touches the base of the sieve. After 24 hours nematode suspension will be collected from the funnel in small beakers. Nematode suspension will be concentrated, if desired by decanting extra water through 350 mesh sieve. Counting of the nematodes will be done under stereoscopic microscope taking 1ml suspension in Peter's 1ml counting slide.

For endoparasitic nematodws, roots will be thoroughly washed till all adhered soil particles are removed. Roots will be cut into pieces of 1-1.5cm length. Out of these, 5g pieces will be transferred into Warring blender containing about 100ml water, will be macerated for

5 seconds or more. Suspensions which will contain macerated root piece and nematodes, will be poured over coarse sieve (50 mesh) mounted with tissue paper, placed in an extraction dish containing enough water. After 48 hours, filter will be removed and the suspension will be observed. In case of sedentary endoparasites, suspension containing macerated roots will diredtly be pou red 2-3 times over coarse sieve and the filterate will be observed.

Killing and Fixing:

Nematodes suspension obtained from Baermann funnel will be collected in 50ml beaker and left undistributed for 4-5 hours, allowing the nematodes to settle at the bottom. Excess of water will be decanted through 350 mesh sieve and thus a concentrated nematode suspension will be obtained. An equal amount (by volume) of boiling double strength F.A. 4:1 will be added to the nematode suspension. In this way nematodes will be killed and fixed simultaneously. Fixed nematodes will be stored in small plastic tubes. The composition of double strength F.A. 4:1 is given below:-

Formaline (Formaldehyde 40%)	= 4 ml
Glacial acetic acid	= 1 ml
Distilled water	= 48ml

Mounting and sealing:

Permanent slides of nematodes will be prepared following the method describe by Siddiqi (1964). Fixed specimens will be transferred from fixative to lactophenol and finally to dehydrated glycrine. Specimens from dehydrated glycerine will be transferred with the help of bamboo splinter in to a drop of dehydrated glycerine on to the glass slide. Nematode will be settled in the middle of the glycerin drop. Three small glass wool pieces of equal thickness corresponding the thickness of the nematodes will be kept at three points in the periphery of glycerine to provide support for a cover glass (No. o, 19mm diameter). Sealing will be done by putting small drop of glyceel at three points along the edge of cover glass and will be ranged with glyceel after 15-25 minutes on a turn table. Second and third coating of glyceel will be applied after 30 minutes with 1 hours interval.

Raising of pure population:

Hoplolaimus indicus, Helicotylenchus retusus and Tylenchorhynchus brassicae will be isolated from various soil samples separately. The method of isolation is given on page 13 . Seedlings of maize (Zea mays.),

tomato (Lycopersicon esculentum Mill.) and cauliflower (Brassica oleracea var. Botrytis L.) raised in 12" clay pots containing autoclaved soil-sand-compost mixture in the ratio of 3:2:1 will be inoculated with the above nematodes respectively. They will multiply on these hosts which will serve as stock culture for subsequent studies.

Studies on Ecological Stresses:

Throughout the studies, 3 week old seedlings of maize, tomato and cauliflower, raised in 6" pots containing soil-sand-compost mixture in the ratio of 3:2:1 will be inoculated with the required nematodes. In case of soil-type studies, this soil will not be used. After 4 months (3 months in case of moisture level study) nematodes will be isolated, processed and stored in plastic tubes. The technique for isolation and processing shall be the same as mentioned on page 13&15.

Various levels of population 100, 10,000 and 1,00000; sex ratio (males/females) 10/90, 50/50 and 90/10; soil type (found in and around Aligarh) Ganga loamy sand, Aligarh loamy sand, Aligarh loam, and Yamuna silty clay loam will be employed. For moisture studies, seedlings will be raised in glazed crocks containing autoclaved soil

of known water holding capacity. A constant moisture level of 10%, 30% and 40% will be maintained by adding requisite amount of water or drying the pots below water holding capacity. Surface of pots will be covered with cellophane sheets to check the loss of water through evaporation. Pots will be weighed at regular interval and requisite amount of water will be added to maintain the desired moisture. To study the influence of host, Hoplolaimus indicus from maize will be transferred to grapes (Vitis vinifera L.), cotton (Gossypium arboreum L.), sugarcane (Saccharum afficinarum L.), tobacco (Nicotiana tabacum L.), tomato (Lycopersicon esculentum L.) , onion (Allium cepa L.), and gram (Cicer arietenum L.); Helicotylenchus retusus from tomato to sugarcane, grapes, tobacco, chilli (Capsicum annum L.), mustard (Brassica nigra L.(koch.)), onion, okra (Abelmoschus esculentus (L.) Moench. and luffa (Luffa cylindrica(L.) Roem.); and Tylenchorhynchus brassicae from cauliflower to mustard, garlic, castor (Ricinus communis L.), chilli, spinach (Spinacea oleracea L.), luffa, tomato and cabbage (Brassica oleracea var. capitata L.). For cross inoculation, H. indicus , H. retusus and T. brassicae from these plants will be transferred back to maize, tomato and cauliflower respectively.

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